Effect of the addition of phytomix-3+ mangosteen on antioxidant activity, viability of lactic acid bacteria, type 2 diabetes key-enzymes, and sensory evaluation of yogurt

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ABSTRACT

Phytomix-3 was prepared from the mixture of Lycium barbarum, Momordica grosvenori and Psidium guajava leaves and added together with Garcinia mangostana pulp and pericarp. The effect of phytomix-3+ mangosteen (Ph-3+M) enriched yogurt was studied for peptide content, inhibitory activities on α-amylase, α-glucosidase, the viability of S. thermophilus and Lactobacillus spp. during 0, 7 and 14 days of storage, both before (pre-) and after (post-) simulated gastrointestinal digestion. Antioxidant activity (DPPH assay), total phenolic content and sensory evaluation were also investigated during the storage period. Ph-3+M yogurt showed significantly higher (p < 0.05) antioxidant activity and TPC compared to plain yogurt during storage. The highest peptide concentrations of pre- and post-digested Ph-3+M yogurt was shown on day 7 of storage. Ph-3+M yogurt displayed strong inhibition on α-glucosidase and mild inhibition on α-amylase inhibitory activities. Ph-3+M yogurt enhanced the viability of S. thermophilus and Lactobacillus spp. In conclusion, Ph-3+M yogurt could provide beneficial effects as a functional food.

1. Introduction

Diabetes is a disease characterized by high blood sugar level (glucose) that result from the failure of the body to produce enough insulin or unable to respond properly to the insulin that had been produced by the pancreas (Shori, 2015a and b). Glucose is an essential nutrient that provides energy for the body to function well. Carbohydrate is broken down into smallest sugar (for example glucose) inside the small intestine and the glucose is absorbed by the intestinal cells into the bloodstream and carried out to all cells in the body that utilized it (Shori, 2015a and b). However, glucose needs insulin to aid in its transport into the cells because it cannot enter cells alone by itself. Consequently, in the absence of insulin, a sudden rise in blood glucose level occurred due to hydrolysis of starch by pancreatic α-amylase and uptake of glucose by α-glucosidase (Shori & Baba, 2013). Thus, an effective strategy for type-2 diabetes management is to inhibit these key enzymes (Baba, Najarian, Shori, Lit, & Keng, 2014).

Yogurt is a dairy product produced from the fermentation of milk by Lactobacillus bulgaricus and Streptococcus thermophilus (Alenisan, Alqattan, Tolbah, & Shori, 2017). It is regarded as a functional food because it contains live LAB, the probiotics that is beneficial for human intestines (Shori, 2016). Yogurt is also rich in calcium, protein, riboflavin, vitamin B6 and vitamin B12 (Muniandy, Shori, & Baba, 2017).

Herbs are normally used for medicine, food flavoring and also as fragrant properties (Emmanuel, Shori, & Baba, 2016). The previous study showed that several dietary herbs, spices, fruits and vegetables which constitute by phenolic phytochemicals can result in high antioxidant activity and possess therapeutic properties which are beneficial for the management of type-2 diabetes (Shetty, Clydesdale, & Vettem, 2005). Lycium barbarum, Momordica grosvenori, Psidium guajava leaf and Garcinia mangostana have been selected for the present study due to the variety of therapeutic properties in these herbs (Shori, 2015a). L. barbarum has shown potential health benefit effects i.e. powerful antioxidant activity, blood glucose levels regulation, blood pressure regulation (Zou, Zhang, Yao, Niu, & Gao, 2010; Baba et al., 2014). M. grosvenori has potential against the diabetic effect, anti-carcinogenic and was used as a natural sweetener (Takasaki et al., 2003) while P. guajava leaf has a beneficial effect as a powerful antioxidant (Soman, Rajamanickam, Rauf, & Indira, 2013). P. guajava contains copious amounts of phenolic phytochemicals which inhibit peroxidation reaction in the living body and therefore can be expected to prevent various chronic diseases such as diabetes, cancer, and heart-disease (Soman et al., 2013). It was reported that the leaves of P. guajava contain an
that 60 ml of dH2O were used to replace the water herbal extract. The mixture was prepared in the same method as the herb’s yogurt with the exception that 14 g full cream milk powder to adjust the milk solid content. The mixture then was put in the container and incubated at 41 °C and stored for 2 h. The addition of 1:2:3 ratio together with pulp and pericarp using 3:2 ratio (60 ml) into 510 ml of pre-heated full cream milk, followed by the e addition of water herbal extract (1.0 ml) was transferred to the test tube and 1.0 ml of 95% ethanol together with 5 ml of dH2O were added. Then, 0.5 ml of 50% Folin-Ciocalteu reagent (Sigma Aldrich, USA) was added to the mixture. After 5 min, 1.0 ml of 5% Na2CO3 was added and the mixture and was left for 1 h at room temperature. Absorbance was read at 725 nm and the absorbance values were converted to total phenolics by referring to gallic acid standard curve. Gallic acid standard curve was prepared with a various concentration of gallic acid (5-60 μg/ml) in ethanol.

2.6. Total phenolic assay

Total phenolics content were determined by applying an assay that had been modified from Muniandy et al. (2016). Yogurt extract (1.0 ml) was transferred to the test tube and 1.0 ml of 95% ethanol together with 5 ml of dH2O were added. Then, 0.5 ml of 50% Folin-Ciocalteu reagent (Sigma Aldrich, USA) was added to the mixture. After 5 min, 1.0 ml of 5% Na2CO3 was added and the mixture and was left for 1 h at room temperature. Absorbance was read at 725 nm and the absorbance values were converted to total phenolics by referring to gallic acid standard curve. Gallic acid standard curve was prepared with a various concentration of gallic acid (5-60 μg/ml) in ethanol.

2.7. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay

The DPPH radical scavenging assay was carried out according to Muniandy et al. (2016) with slight modification. 3 ml of 60 mM DPPH (dissolved in ethanol; Sigma Aldrich, USA) was added with 250 μl of yogurt extracts and allowed to stand at room temperature (25 °C) for 1 h. The absorbance was recorded at 517 nm against control, which contained 250 μl of ethanol instead of the extract. The % of inhibition was calculated by using following formula:

\[
\text{Inhibition} \% = \frac{\text{AC} - \text{AS}}{\text{AC}} \times 100\%
\]

\[
\text{AC} = \text{Absorbance of control}
\]

\[
\text{AS} = \text{Absorbance of sample}
\]

2.8. In vitro gastrointestinal model

2.8.1. Preparation of gastric and duodenum juices

The gastric and duodenum solutions were freshly prepared according to the protocols described by Shori and Baba (2015). To simulate the in vivo saliva, 100 ml of a sterile electrolyte solution (6.2 g/l NaCl, 2.2 g/l KCl, 0.22 g/l CaCl2, 1.2 g/l NaHCO3) was mixed with 10 mg lysozyme from chicken egg white (Sigma Aldrich, USA) to obtain a final concentration of 100 ppm. To simulate the stomach environment (gastric juice), 0.3% of the electrolyte solution was added to pepsin (P7000; Sigma Aldrich, USA) and the pH was adjusted to 3 using 5 M HCl. To simulate the intestinal digestion (duodenum juice), the electrolyte solution (6.4 g/l NaHCO3, 0.239 g/l KCl, 1.28 g/l NaCl) mixed with 0.3% bile salts (B8631; Sigma Aldrich, USA) and 0.1% (v/w) pancreatin (P3292; Sigma Aldrich, USA) and adjusted to pH 7.2 by using 5 M NaOH.

2.8.2. Simulation of gastrointestinal digestion

Yogurt samples were taken out and diluted with the artificial saliva solution in the ratio of 1:1 and incubated at 37 °C for 5 min. The samples were further diluted with an artificial gastric fluid solution in the ratio of 3:5 and incubate again at 37 °C for 1 h before 30 ml of samples were taken out for analysis (α-amylase and α-glucosidase inhibition assays). The remaining solutions from stomach section were further diluted with artificial duodenal secretion in the ratio of 1:4 and were incubated again at 37 °C for 2 h where 30 ml of the samples were taken out for analysis (α-amylase and α-glucosidase inhibition assays) after
1 h and 2 h incubation of intestinal digestion. All samples after 3 h gastrointestinal digestion (post-digestion) were analysis for the viability of *S. thermophilus* and *Lactobacillus* spp. and peptide concentration. All samples were manually agitated and stirred intermittently during the incubation time in order to ensure adequate enzymatic digestion to mimic gastrointestinal movement. The samples that were taken out for analysis were extracted by using a similar method as the extraction of yogurt water extract (section 2.3) in order to remove excess proteins, electrolytes and other impurities (Shori & Baba, 2015).

### 2.9. Microbial viable cell count

#### 2.9.1. Enumeration of *Streptococcus thermophilus*

*S. thermophilus* was enumerated using spread plate method. Samples were serially diluted to the desired dilution factor (10⁻²) using peptone water buffer. Sterilized M17 media then was poured onto a clean sterilized petri dish and this agar was allowed to solidify. Then, 0.1 ml of diluted sample was transferred onto the agar surface and evenly distributed in three different directions using a sterile glass spreader. The plate was then incubated at 37 °C for 2 days. Finally, the number of colonies formed after incubation was expressed as colony forming units per milliliter sample (CFU/ml) using the following formulation:

\[ CFU/\text{ml} = \frac{\text{No. of colonies formed} \times \text{dilution factor of sample}}{0.1 \text{ ml of sample}} \]

*CFU*: Colony forming unit

#### 2.9.2. Enumeration of *Lactobacillus* spp

*Lactobacillus* spp was enumerated using pour plate method. Samples were serially diluted to the desired dilution factor (10⁻²) using peptone water buffer. The MRS medium was maintained at molten state (45-50 °C). Then, 1.0 ml of diluted sample was transferred to a sterile petri dish and 10 ml of sterile MRS medium was poured onto the petri dish and this mixture was mixed evenly. Care was taken to ensure that the entire surface of the plate was fully covered by the first layer of medium. The second layer of MRS media was poured on the solidified first layer of MRS agar. The petri dish was sealed with parafilm after the second layer of MRS has solidified and incubation was carried out at 37 °C for 2 days. The number of colonies formed was counted and the viable cell count in the sample was expressed as colony forming units per milliliter sample (CFU/ml) using the following formulation:

\[ CFU/\text{ml} = \frac{\text{No. of colonies formed X dilution factor of sample}}{1.0 \text{ ml of sample}} \]

#### 2.10. O-phthalaldehyde (OPA) assay

Yogurt extracts (30 μl, containing 5-100 μg protein) were added with 1.0 ml of OPA reagent (25 ml of 100 mM sodium tetraborate, 2.5 ml of 20% (w/w) sodium-dodecyl-sulphate, 40 mg OPA dissolved in 1 ml of methanol and 100 μl of β-mercaptoethanol top up with dH2O until final volume is 50 ml) in 1.5 ml cuvette. The cuvette was then covered with parafilm prior to several times inversion in order to homogenize and facilitate the mixing of OPA reagent and samples. The mixture was then left at room temperature for 2 min. Absorbance was read at 340 nm and the peptide concentration was determined from Tryptone standard curve using different concentrations (Church, Swaisgood, Porter, & Catignani, 1983; Shori, 2013a, pp. 202–208).

#### 2.11. α-Amylase inhibition assay

Yogurt extracts (500 μl) were mixed with 1.0 ml of 0.02 M sodium phosphate buffer, pH 6.9 with 0.006 M sodium chloride containing 0.5 mg/ml α-amylase (1080; Sigma Aldrich, USA) solution and this solution were incubated at 37 °C for 10 min (Shori & Baba, 2013). Upon pre-incubation, 500 μl of a 1% starch solution in 0.02 M sodium phosphate buffer, pH 6.9 with 0.006 M sodium chloride was added to each tube at a pre-determined time interval (every 20 s). The reaction mixtures were then incubated again at 37 °C for 10 min. To terminate the reactions, 1.0 ml of 2% dinitrosalicylic acid (D0550, Sigma Aldrich, USA) color reagent was added to the mixture. The test tubes were then incubated in a boiling water bath for 8-9 min. 1.0 ml of tartrate solution (18.2% w/v; S2377 Sigma Aldrich, USA) was then added into each tube and upon cooling to room temperature (approximately 2-3 min) and the reaction mixture was diluted with 10 ml of dH2O. Absorbance was read at 540 nm. The readings were compared to a control using 500 μl of buffer solution in place of the extract. The formula to calculate enzyme inhibition is as follows:

\[ \text{α-Amylase inhibition %} = \left( \frac{\text{AS} - \text{AC}}{\text{AC}} \right) \times 100\% \]

\[ \text{AC} = \text{Absorbance of control.} \]

\[ \text{AS} = \text{Absorbance of sample.} \]

#### 2.12. α-Glucosidase inhibition assay

The α-glucosidase inhibition assay was performed as described by Shori and Baba (2013). Potassium phosphate buffer (1.0 ml, 0.8 M, pH 6.90) containing α-glucosidase (G5003; Sigma Aldrich, USA) solution (0.15 U/ml) was added into 50 μl of sample extract and the solution was incubated in a water bath (37 °C) for 10 min. After incubation, 500 μl of 5 mM p-nitrophenyl-α-glucopyranoside solution (N1377; Sigma Aldrich, USA) in 0.1 M potassium phosphate buffer (pH 6.90) was added to each tube at pre-determined time intervals (every 30 s), and the absorbance at 405 nm was taken (time = 0 min). The reaction mixtures were further incubated at 37 °C for 10 min. After 10 min of incubation, absorbance readings were taken again at 405 nm. The readings were compared to a control using 500 μl of buffer solution in place of the extract. Readings for both control and samples at 10 min were deducted from the readings at 0 min. The α-glucosidase inhibitory activity was expressed as follows:

\[ \text{α-Glucosidase inhibition %} = \left( \frac{\text{AC} - \text{AS}}{\text{AC}} \right) \times 100\% \]

\[ \text{AC} = \text{Absorbance of control.} \]

\[ \text{AS} = \text{Absorbance of sample.} \]

#### 2.13. Sensory evaluation

0, 7, and 14 days yogurt samples were assessed by 30 untrained assessors (Shori & Baba, 2012). Sensory parameters which include texture (presence of whey), consistency (graininess, lumpiness, and firmness), sourness, sweetness, bitterness, overall aroma and overall preference were evaluated using a score rating of 1–10 points. The assessors were briefed on how to rate the yogurt samples before the assessment session to give a better understanding of the parameters which were being assessed. All the sensory parameters were analyzed with respect to a reference sample which includes the plain and phytomix-3+ mangosteen yogurt.

#### 2.14. Statistical analysis

The experiment was carried out using three different batches of yogurt (n = 3). Data were expressed as mean ± SE. The statistical analysis was performed using one-way analysis of variance (ANOVA, SPSS 14.0), followed by Duncan’s post hoc test for mean comparison. The criterion for statistical significance was p < 0.05.

### 3. Results and discussion

#### 3.1. Effect of phytomix-3+ mangosteen on total phenolic content (TPC) of yogurt

The presence of phytomix-3+ mangosteen increased significantly (p < 0.05) TPC in yogurt ranged from 40 to 41 μg/g compared to plain
yogurt (15-17 μg/g) during 14 days of storage (Fig. 1). The combination of phytomix-3+ mangosteen contributed to a high amount of phenolic compounds. This could be due to the presence of phenolic phytochemicals in these herbs (Shetty et al., 2005). This result was similar to the TPC of Allium sativum, white tea, and soybean yogurt during 14 days of storage which related to active compounds derived from Allium sativum (Muniandy et al., 2016; Shori et al., 2013; Shori & Baba, 2014b). Further study is needed to characterize the phenolic profiles of phytomix-3+ mangosteen yogurt. Phenolic phytochemicals are plant secondary metabolites which constitute one of the most abundant groups of natural metabolites. The plants synthesize these compounds for their protection against biological and environmental stress (Shetty et al., 2005). Elevation of TPC in food is commonly associated with increased antioxidant activities (Shori & Baba, 2013).

The degradation of milk proteins during milk fermentation resulted in an increase in TPC. This is because the release of phenolic amino acids and non-phenolic compounds such as sugars and proteins may interfere during the total phenolic assessment (Ainsworth & Gillespie, 2007).

3.2. Effect of phytomix-3+ mangosteen on antioxidant activity of yogurt

Plain yogurt showed antioxidant activity ranged between 17 and 19% during 14 days of storage (Fig. 2). However, the presence of phytomix-3+ mangosteen increased (p < 0.05) antioxidant activity in yogurt. The antioxidant activity was ranged from 60 to 62% in the first week of storage followed by reduction (p < 0.05) to 54% on day 14 of storage (Fig. 2). Previous studies reported lower DPPH values ranged from 35% to 22% and 30%-50% for Cinnamomum verum and Azadirachta indica yogurts, respectively during 7 days of storage (Shori and Baba, 2011, 2013). The DPPH inhibition activity is closely related to the phenolic content (Baba et al., 2014). Therefore, antioxidants in phytomix-3+ mangosteen yogurt were found to be positively correlated with TPC (R² = 0.977; data not shown). The present study gives rise to the possibility of the prolonged shelf life of yogurt in the presence of phytomix-3+ mangosteen as it recorded high and retained antioxidant capacity throughout the 14 days of storage.

3.3. Effect of phytomix-3+ mangosteen on the viability of S. thermophilus and Lactobacillus spp of yogurt

There was no significant difference in the viable cell counts of S. thermophilus of pre-digested yogurt during 14 days of storage which ranged from 8.5 to 9.2 long10 CFU/ml and 7.9-8.4 long10 CFU/ml for phytomix-3+ mangosteen and plain yogurt, respectively (Table 1). A similar result was almost observed for the viability of Lactobacillus spp for both types of yogurt ranged from 6.5-7.4 long10 CFU/ml during 14 days of storage (Table 1). Post-digested yogurt reduced the viability of S. thermophilus and Lactobacillus spp in both types of yogurt (Tables 1 and 2). However, the presence of phytomix-3+ mangosteen significantly increased (1 long10 CFU/ml; p < 0.05) the viability of S. thermophilus in post-digested yogurt compared to plain yogurt during 14 days of storage. The highest viability of S. thermophilus and Lactobacillus spp was found on day 7 of storage for both plain and phytomix-3+ mangosteen yogurt (Tables 1 and 2).

Table 1

<table>
<thead>
<tr>
<th>Viability of S. thermophilus and Lactobacillus spp. Sections Yogurt Samples</th>
<th>14 day</th>
<th>7 day</th>
<th>0 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre Phytomix-3+ Plain</td>
<td>8.9 ± 0.07</td>
<td>9.2 ± 0.09</td>
<td>8.5 ± 0.07</td>
</tr>
<tr>
<td>Post Mangosteen</td>
<td>6.2 ± 0.06</td>
<td>7.1 ± 0.12</td>
<td>5.0 ± 0.09</td>
</tr>
<tr>
<td>Pre</td>
<td>8.1 ± 0.06</td>
<td>8.4 ± 0.06</td>
<td>7.9 ± 0.12</td>
</tr>
<tr>
<td>Post</td>
<td>5.3 ± 0.09</td>
<td>6.1 ± 0.06</td>
<td>4.2 ± 0.06</td>
</tr>
<tr>
<td>Viability of Lactobacillus spp. (log10 CFU/ml) Pre Plain</td>
<td>6.9 ± 0.03</td>
<td>7.4 ± 0.06</td>
<td>6.5 ± 0.12</td>
</tr>
<tr>
<td>Post Mangosteen</td>
<td>4.9 ± 0.03</td>
<td>5.5 ± 0.06</td>
<td>4.8 ± 0.06</td>
</tr>
<tr>
<td>Pre</td>
<td>6.5 ± 0.03</td>
<td>7.0 ± 0.03</td>
<td>6.9 ± 0.06</td>
</tr>
<tr>
<td>Post</td>
<td>4.3 ± 0.09</td>
<td>5.1 ± 0.06</td>
<td>4.0 ± 0.09</td>
</tr>
</tbody>
</table>

*Before (pre-) gastrointestinal digestion = during storage at 0, 7, & 14 days. After (post-) gastrointestinal digestion = during 3 h of digestion (gastric digestion = 1 h; intestinal digestion = 2 h).

Table 2

<table>
<thead>
<tr>
<th>Peptide concentration (mg/ml) Sections Yogurt Samples</th>
<th>14 day</th>
<th>7 day</th>
<th>0 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre Phytomix-3+ Plain</td>
<td>33.66 ± 0.52</td>
<td>36.14 ± 0.32</td>
<td>27.28 ± 1.35</td>
</tr>
<tr>
<td>Post Mangosteen</td>
<td>57.79 ± 1.40</td>
<td>89.03 ± 1.94</td>
<td>55.41 ± 1.79</td>
</tr>
<tr>
<td>Pre</td>
<td>27.73 ± 1.12</td>
<td>28.13 ± 1.29</td>
<td>24.20 ± 0.48</td>
</tr>
<tr>
<td>Post Plain</td>
<td>57.73 ± 2.52</td>
<td>64.23 ± 0.81</td>
<td>54.39 ± 2.12</td>
</tr>
</tbody>
</table>

*Before (pre-) gastrointestinal digestion = during storage at 0, 7, & 14 days. After (post-) gastrointestinal digestion = during 3 h of digestion (gastric digestion = 1 h; intestinal digestion = 2 h).
present study, the presence of phytomix-3+ mangosteen in yogurt increased *S. thermophilus* and *Lactobacillus* spp. counts compared to plain yogurt. However, the viability of both *S. thermophilus* and *Lactobacillus* spp. was drastically reduced as yogurt subjected to gastrointestinal digestion compared to undigested yogurt. This could be due to the nature of *S. thermophilus* which is acid sensitive (Shori & Baba, 2012) and unable to resist the bile salts that cause the low rate of viability. *S. thermophilus* can grow well at pH range 5.5–6.2 but decreased as pH approaches 4.1 (Shori & Baba, 2015). The normal yogurt cultures (*Lactobacillus* spp. and *S. thermophilus*) are not bile resistant or acid tolerant and thus cannot survive in the intestinal tract (Shori & Baba, 2012).

### 3.4. Effect of phytomix-3+ mangosteen on peptide concentration of yogurt

Peptide concentrations for pre-digested fresh (0 day) plain and phytomix-3+ mangosteen yogurt were 24.20 ± 0.48 and 27.28 ± 1.35 mg/g/ml, respectively (Table 2). The highest peptide concentrations of pre-digested phytomix-3+ mangosteen and plain yogurts were shown on day 7 of storage (36.14 ± 0.32 and 28.13 ± 1.29 mg/g/ml; respectively). These followed by a non-significant decline on last day of storage (Table 2). There were no significant differences in peptide concentration of post-digested fresh yogurt in both presence and absence of phytomix-3+ mangosteen (Table 2). However, post-digested phytomix-3+ mangosteen yogurt showed the highest peptide concentrations (89.03 ± 1.94 mg/ml) on day 7 of storage while plain yogurt was 64.23 ± 0.81 mg/ml. Prolonged storage for another week reduced significantly (p < 0.05) peptide concentrations to a similar number for both types of post-digested yogurt (Table 2).

OPA has been widely used for the assay of amines group of amino acid, peptides, and protein (Medina Hernandez, 1990). It can detect the smallest amount of protein and peptide that may be formed during the proteolysis. The proteolytic activity was largely carried out by *L. bulgaricus* (Shori & Baba, 2012). The highest peptide concentrations of phytomix-3+ mangosteen yogurt both pre- and post-digested on day 7 of storage could be due to the greater viability of LAB which enhanced the proteolytic activity by the LAB in yogurt. This assumption was supported by higher viable cell counts of *Lactobacillus* spp. in phytomix-3+ mangosteen yogurt (Table 1). Extension of the storage to 14 days resulted in a decrease in peptide concentrations of pre- and post-digested yogurt possibly due to the low viability of LAB (Tables 1 and 2). Lower peptide concentrations (22-17 mg/g) have been shown in *Azadirachta indica* yogurt pre-digested (Shori & Baba, 2013). Similarly, the peptide concentrations pre-digested of *A. sativum* and *C. verum* yogurt were ranged between 0.24 and 0.26 mg/g and 0.14–0.17 mg/g, respectively. *A. sativum*-fish collagen-yogurt and fish collagen-yogurt were shown highest peptide concentrations (0.34 ± 5.3 and 0.28 ± 2.0 mg/g); respectively) on day 7 of storage (Shori, Baba, & Chauh, 2013).

The increasing number of peptide has been identified in milk protein hydrolysates and also in fermented dairy products such as yogurt (Kilara & Panyam, 2003). Bioactive peptides can be released from their parent protein via three ways, (1) enzymatic hydrolysis by digestive enzyme, (2) fermentation of milk with proteolytic starter cultures and (3) proteolysis by the enzyme derived from microorganisms or plants. Each or combination of this steps has been reportedly shown to affect the production of short functional peptides (Korhonen & Pihlanto, 2006).

In comparison to post-digestion yogurt, the peptide concentration was greater than pre-digest. This suggests the release of some of the bioactive peptides by the enzymic hydrolysis of the proteolytic enzyme (Korhonen, 2009). Proteolysis of milk protein by proteolytic LAB usually associated with the degradation of the protein by proteinaises into polypeptides, which can be further degraded to small molecular weights peptides and free amino acids by the peptidases (Christensen, Dudley, Pederson, & Steele, 1999). Besides, the presence of enzyme protease inside the pancreatin of the digestive system enhanced the proteolysis of the peptide. This observation is in agreement with our findings (Table 2) since the amount of peptide was greatly increased after simulated gastrointestinal digestion.

### 3.5. Effects of phytomix-3+ mangosteen on α-glucosidase and α-amylase inhibition of yogurt

The presence of phytomix-3+ mangosteen in yogurt (pre-digested) enhanced (p < 0.05) the inhibition of α-glucosidase and α-amylase activity compared to plain yogurt during 14 days of storage (Table 3). The highest α-glucosidase inhibition was shown on day 7 of storage for both phytomix-3+ mangosteen and plain yogurts (16.32 ± 1.32% and 11.51 ± 0.63%; respectively). Similarly, phytomix-3+ mangosteen yogurt showed the highest α-amylase inhibitory activity

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**Table 3**

α-Glucosidase and α-Amylase inhibition (%) before (pre-) and after (post-) gastrointestinal digestion of phytomix-3+ mangosteen and plain yogurt over 14 days of storage.

<table>
<thead>
<tr>
<th>Day</th>
<th>α-Glucosidase Inhibition %</th>
<th>α-Amylase inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI sections</td>
<td>Yogurt samples</td>
<td></td>
</tr>
<tr>
<td>14 day</td>
<td>7 day</td>
<td>0 day</td>
</tr>
<tr>
<td>9.22 ± 0.63</td>
<td>16.32 ± 1.32</td>
<td>15.20 ± 0.60</td>
</tr>
<tr>
<td>3.88 ± 0.60</td>
<td>5.38 ± 0.11</td>
<td>4.88 ± 0.26</td>
</tr>
<tr>
<td>3.20 ± 0.44</td>
<td>3.84 ± 0.65</td>
<td>5.16 ± 0.22</td>
</tr>
<tr>
<td>6.21 ± 0.47</td>
<td>11.51 ± 0.63</td>
<td>11.29 ± 0.79</td>
</tr>
<tr>
<td>2.20 ± 0.44</td>
<td>3.84 ± 0.65</td>
<td>5.16 ± 0.22</td>
</tr>
<tr>
<td>39.22 ± 0.44</td>
<td>55.68 ± 0.83</td>
<td>48.53 ± 0.26</td>
</tr>
<tr>
<td>23.93 ± 0.54</td>
<td>27.29 ± 0.52</td>
<td>33.94 ± 1.93</td>
</tr>
<tr>
<td>23.93 ± 0.54</td>
<td>27.29 ± 0.52</td>
<td>33.94 ± 1.93</td>
</tr>
<tr>
<td>50.51 ± 2.01</td>
<td>53.31 ± 0.63</td>
<td>49.97 ± 2.19</td>
</tr>
<tr>
<td>34.53 ± 2.89</td>
<td>39.04 ± 1.38</td>
<td>39.99 ± 1.49</td>
</tr>
<tr>
<td>25.19 ± 2.89</td>
<td>29.57 ± 0.94</td>
<td>32.97 ± 1.50</td>
</tr>
<tr>
<td>23.93 ± 0.54</td>
<td>27.29 ± 0.52</td>
<td>33.94 ± 1.93</td>
</tr>
<tr>
<td>45.59 ± 2.74</td>
<td>49.62 ± 0.39</td>
<td>48.36 ± 1.91</td>
</tr>
<tr>
<td>29.97 ± 1.03</td>
<td>33.64 ± 1.63</td>
<td>31.99 ± 0.48</td>
</tr>
<tr>
<td>20.82 ± 1.20</td>
<td>24.64 ± 1.50</td>
<td>28.92 ± 2.51</td>
</tr>
<tr>
<td>18.72 ± 0.78</td>
<td>22.79 ± 0.62</td>
<td>28.73 ± 3.08</td>
</tr>
</tbody>
</table>

*Before (pre-) gastrointestinal digestion = during storage at 0, 7, & 14 days. After (post-) gastrointestinal digestion (Gastric digestion = 1 h; Intestinal digestion = 1 & 2 h).*
Phytomix-3+ mangosteen yogurt showed higher score in terms of sweetness (5.6) and aroma (6.7) compared to plain-yogurt (2.3 and 4.5; for sweetness and aroma respectively). However, the texture and consistency had almost the same score for both types of yogurt (Fig. 3). Moreover, sourness and bitterness were lower in phytomix-3+ mangosteen yogurt compared to plain yogurt; indicated that the taste was considered more acceptable for phytomix-3+ mangosteen yogurt.

Food additives are commonly added into dairy products (Shori & Baba, 2012, 2013; Shori, Baba, & Solear, 2016) because they attributed important sensory characteristics such as taste, appearance, consistency and prolong the shelf life of the dairy products (Nakazawa et al., 1992). The addition of phytomix-3+ mangosteen into yogurt showed greater preference by consumers than plain yogurt. However, since phytomix-3+ mangosteen yogurt has potential to be used as suitable health supporting dietary product, several important characteristics such as texture and the shelf life of this yogurt need to be improved in order to fulfill consumer acceptance.

4. Conclusion

The present study showed that yogurt enriched with phytomix-3+ mangosteen can inhibited over 50% of free radical scavenging-linked antioxidant activity. In addition, phytomix-3+ mangosteen yogurt displayed strong inhibition toward α-glucosidase and mild inhibition of α-amylase inhibitory activities after exposure to simulated gastrointestinal digestion. Phytomix-3+ mangosteen yogurt (pre- and post-digested) enhanced the viability of S. thermophilus and Lactobacillus spp. compared to plain yogurt during 14 days of storage. The presence of phytomix-3+ mangosteen in yogurt has some influence on the sensory characteristics. Thus, phytomix-3+ mangosteen yogurt has great potential to be suitable functional food rich in antioxidant and to help manage type-2 diabetes by inhibiting the key enzymes linked to this disease. Further studies are needed to evaluate phenolic compounds and antioxidant activity of phytomix-3+ mangosteen yogurt during 0, 7 and 14 days of storage, both before (pre-) and after (post-) simulated gastrointestinal digestion as well as characterize the phenolic and antioxidant profiles of the sample.

References


